Claims

1. EPO composition,
wherein
it is composed essentially of glycosylated EPO molecules which contain a proportion of at least 75 % of tetraantennary structures relative to the total number of N-linked carbohydrate chains.

2. EPO composition,
wherein
it is composed of glycosylated EPO molecules which contain an average proportion of at least 75 % of tetraantennary structures relative to the total number of N-linked carbohydrate chains.

3. EPO composition as claimed in claim 1 or 2,
wherein
the proportion of tetraantennary structures is at least 80 %.

4. EPO composition,
wherein
it is essentially composed of glycosylated EPO molecules which contain a number of on average at least 3.7 N-acetyl-lactosamine units with reference to an N-linked carbohydrate chain of an EPO molecule or on average at least 11.1 N-acetyl-lactosamine units with reference to the total N-glycosylation of an EPO molecule.

5. EPO composition,
wherein
it is composed of glycosylated EPO molecules which contain an average number of on average at least 3.7 N-acetyl-lactosamine units with reference to an N-linked carbohydrate chain or on average at least 11.1 N-acetyl-lactosamine units with reference to the total N-glycosylation of an EPO molecule.

6. EPO composition as claimed in claim 4 or 5,
wherein
the number of N-acetyl-lactosamine units is at least 4.0 with reference to an N-linked carbohydrate chain or 12.0 with reference to the total N-glycosylation.

7. EPO composition
wherein
it is essentially composed of glycosylated EPO molecules which have a value for the product of the average number of N-acetyl-lactosamine units with reference to an N-linked carbohydrate chain of an EPO molecule multiplied by the average sialic acid content per molecule of EPO of at least 43.3 or at least 130 relative to the total N-glycosylation of an EPO molecule.

8. EPO composition
wherein
it is composed of glycosylated EPO molecules which have an average value for the product of the average number of N-acetyl-lactosamine units with reference to an N-linked carbohydrate chain of an EPO molecule

multiplied by the average sialic acid content per molecule of EPO of at least 43.3 or at least 130 with reference to the total N-glycosylation of an EPO molecule.

9. EPO composition as claimed in claim 7 or 8, **wherein** the value of the product is at least 46.7 with reference to an N-linked carbohydrate chain or at least 140 with reference to the total N-glycosylation.

10. EPO composition, **wherein** it has the features of at least two of the claims 1, 2, 4, 5, 7 and 8.

11. EPO composition as claimed in one of the previous claims, **wherein** it comprises a mixture of 2 to 5 isoforms.

12. EPO composition as claimed in claim 11, **wherein** it comprises a mixture of 3 to 4 isoforms.

13. EPO composition as claimed in one of the previous claims, **wherein** it has a specific activity in vivo of at least 175,000 IU/mg protein.

14. EPO composition as claimed in one of the previous claims,
wherein
it has a specific activity in vivo of at least 200,000 IU/mg protein.

15. EPO composition as claimed in one of the previous claims,
wherein
the average sialic acid content per molecule is at least 11.

16. EPO composition as claimed in one of the previous claims,
wherein
the EPO molecules are the product of an expression of exogenous DNA in mammalian cells.

17. EPO composition as claimed in claim 16,
wherein
it is composed of glycosylated EPO molecules from CHO cells in which the proportion of carbohydrate chains with N-acetyl-lactosamine extensions (repeats) relative to the total number of N-linked carbohydrate chains is at least 30 %.

18. EPO composition as claimed in claim 17,
wherein
the value for the product of the proportion of carbohydrate chains with N-acetyl-lactosamine repeats relative to the total number of carbohydrate chains and the proportion of tetraantennary structures relative to the total number of carbohydrate chains is at least 2400.

19. EPO composition as claimed in one of the claims 1 to 15,
wherein
the EPO molecules are the product of an expression of endogenous DNA in human cells.

20. EPO composition as claimed in claim 19,
wherein
the proportion of carbohydrate chains with N-acetyl-lactosamine repeats relative to the total number of carbohydrate chains is at least 10 %.

21. EPO composition as claimed in claim 20,
wherein
the value for the product of the proportion of carbohydrate chains with N-acetyl-lactosamine repeats relative to the total number of carbohydrate chains and the proportion of tetraantennary structures relative to the total number of carbohydrate chains is at least 800.

22. EPO composition as claimed in claim 16 or 19,
wherein
the cells are cultured in a serum-free medium.

23. Pharmaceutical preparation,
wherein
it contains an EPO composition as claimed in one of the claims 1 to 22 as the active substance optionally together with common pharmaceutical diluents, auxiliary substances and carriers.

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24. Process for producing an EPO composition, in particular as claimed in one of the claims 1 to 23, **wherein**
the EPO composition with the desired features is obtained by at least one of the following measures:

(a) selection of a suitable production cell which is able to produce carbohydrate chains with a high proportion of tetraantennary structures or/and N-acetyl-lactosamine units,

(b) selection of suitable culture conditions for the cell culture in order to produce carbohydrate chains with a high proportion of tetraantennary structures or/and N-acetyl-lactosamine units and

(c) separation of undesired components from a known composition of EPO molecules while enriching EPO molecules which contain carbohydrate chains with a high proportion of tetraantennary structures or/and N-acetyl-lactosamine units.

25. Process as claimed in claim 24, **wherein**
measure (b) comprises adding a mixture of at least 2 carbohydrates and preferably at least 3 carbohydrates to the culture medium.

26. Process as claimed in claim 25, **wherein**
a carbohydrate mixture containing glucose or/and mannose or/and galactose is used.

27. Process as claimed in claim 24,
wherein
measure (b) comprises the controlled addition according to needs of nutrients comprising at least one essential amino acid or/and at least one carbohydrate depending on the requirements of the cells.

28. Process as claimed in claim 27,
wherein
the nutrient requirements of the cells are determined dependent on the concentration of glutamine measured in the culture medium.

29. Process as claimed in claim 27 or 28,
wherein
the nutrients are added according to needs over the entire growth phase of the cells.

30. Process as claimed in one of the claims 27 to 29,
wherein
the nutrients comprise a mixture of at least 2 carbohydrates and preferably at least 3 carbohydrates.

31. Process as claimed in one of the claims 24 to 30,
wherein
measure (b) comprises culturing at a temperature between 30 and 35.5°C, preferably between 33 and 35.0°C.

32. Process as claimed in claim 24,
wherein
measure (c) comprises a reverse phase chromatography step at a pH value in the range 6-8.

33. Process as claimed in claim 32,
wherein
acetonitrile, ethanol or isopropanol is used as the eluant.

34. Process as claimed in claim 24,
wherein
measure (c) comprises an affinity chromatography step using triazine dyes.

35. Process as claimed in claim 24,
wherein
measure (c) comprises an affinity chromatography step using lectins.

36. Process for increasing the specific activity of an EPO composition,
wherein
EPO molecules are enriched in the composition which have
(a) a high proportion of tetraantennary carbohydrate structures,
(b) a large number of N-acetyl-lactosamine units
(c) a high value for the product of the number of N-acetyl-lactosamine units and the sialic acid content,
(d) a high proportion of N-acetyl-lactosamine repeats or/and
(e) a high value for the product of the proportion of N-acetyl-lactosamine repeats and the proportion of tetraantennary carbohydrate structures.

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37. Process as claimed in claim 36,
wherein
it is enriched to an average proportion of at least 75 % tetraantennary structures relative to the total number of carbohydrate chains.

38. Process as claimed in claim 36,
wherein
it is enriched to an average number of at least 3.7 N-acetyl-lactosamine units with reference to an N-linked carbohydrate chain of an EPO molecule or on average at least 11.1 N-acetyl-lactosamine units relative to the total N-glycosylation of an EPO molecule.

39. Process as claimed in claim 36,
wherein
it is enriched to a value for the product of the average number of N-acetyl-lactosamine units with reference to an N-linked carbohydrate chain of an EPO molecule multiplied by the average sialic acid content of at least 43.3 or at least 130 relative to the total N-glycosylation of an EPO molecule.

40. Process as claimed in claim 36,
wherein
it is enriched
(a) in the case of EPO from CHO cells to an average proportion of at least 30 % N-acetyl-lactosamine repeats relative to the total number of carbohydrate chains or
(b) in the case of EPO from human cells to an average proportion of at least 10 % N-acetyl-lactosamine repeats relative to the total

number of carbohydrate chains.

41. Process as claimed in claim 36,
wherein
it is enriched
(a) in the case of EPO from CHO cells, to a value of the product of the average proportion of N-acetyl-lactosamine repeats relative to the total number of carbohydrate chains multiplied by the average proportion of tetraantennary carbohydrate structures of at least 2400 or
(b) in the case of EPO from human cells, to a value of the product of the average proportion of N-acetyl-lactosamine repeats relative to the total number of carbohydrate chains multiplied by the average proportion of tetraantennary carbohydrate structures of at least 800.

42. Process as claimed in one of the claims 36-41,
wherein
the enrichment is achieved by one or several of the measures (a), (b) and (c) as claimed in claim 24.

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